Morphology, Distribution and Host Range of the Lucerne Race of *Ditylenchus dipsaci* in New South Wales

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Measurements of *Ditylenchus dipsaci* from *Medicago sativa* (lucerne) from three localities in New South Wales and one in South Australia are given and the morphology of the nematodes described. In surveys of some major lucerne areas in New South Wales, the nematode occurred in 22% of the lucerne crops sampled. Cross inoculation indicated that *D. dipsaci* from *M. sativa* did not reproduce in bulbs of *Narcissus peudonarcissus* (daffodil); *D. dipsaci* from *N. pseudonarcissus* caused some swelling of *M. sativa* seedlings and small numbers were in the seedlings four days after inoculation. Inoculation of seed of 14 different plant species with *D. dipsaci* from lucerne from two locations caused distorted growth of *Allium cepa, Lycopersicon esculentum, M. sativa, Phaseolus vulgaris* and *Pisum sativum. D. dipsaci* was recovered from the stems and leaves of these plants 4 weeks after inoculation but not 10 weeks after.

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INTRODUCTION

The stem nematode, *Ditylenchus dipsaci* Huhn) was first reported on lucerne (*Medicago sativa* L.) in Australia by Noble (1925). Subsequently it has become widespread in the Hunter Valley, New South Wales, where first found, and in the Lachlan-Belubula River districts of New South Wales; it is also a problem in South Australia (Dubé, 1975). Despite the economic importance of its main host, the morphology and bionomics of the stem nematode on lucerne in Australia have not been recorded. This paper reports its measurements and morphology and records studies on its incidence in New South Wales and its host range.

MEASUREMENTS AND MORPHOLOGY

Measurements of races of *D. dipsaci* are influenced by host (Goodey, 1941; Barraclough and Blackith, 1962; Blake, 1962; Metlitzky, 1969) and a "Giant Race" is known (Goodey, 1941). Measurements in standard descriptions (Goodey, 1963; Hooper, 1972) are those of Thorne (1945), who studied specimens collected from Fuller's teasel (*Dipsacus sativus* (L.) Honck.), the type host, near Molalla, Oregon, U.S.A. (Thorne, 1961). Thorne (1945) described specimens from teasle, Goodey (1963) and Hooper (1972) also provided brief descriptions of the nematode and Wu (1958; 1960; 1967) studied the reproductive systems of *Ditylenchus* species.

Sixteen females and 16 males of *D. dipsaci* from lucerne plants collected from Wagga Wagga, N.S.W., Whittingham, Scotts Flat (near Singleton) N.S.W. and Langhorne Creek, South Australia were measured (Table 1) and their morphology studied.

TABLE 1

Measurements (means and ranges, n = 16) of *Ditylenchus dipsaci* from lucerne in Australia.

Locality	L (μm)	a	b	c	V	T
Langhorne Creek, South Australia	1146	43	6.2	14	79	—
	980-1274	36-49	5.4-6.9	13-17	76-82	—
	1104	44	6.2	14	—	61
	993-1231	36-51	5.5-7.7	13-16	—	45-74
Scotts Flat, New South Wales	1264	47	6.4	16	80	—
	1153-1410	40-55	5.9-7.1	14-18	69-89	—
	1168	50	5.7	15	—	60
	997-1307	40-58	5.0-7.0	13-16	—	49-67
Whittingham, New South Wales	1132	44	6.5	14	80	—
	1009-1274	38-55	5.5-7.3	11-16	77-83	—
	1142	46	6.1	13	—	61
	1037-1344	38-57	5.1-8.4	15-17	—	53-73
Wagga Wagga, New South Wales	1324	49	7.1	15	80	—
	1165-1448	43-65	6.2-7.6	13-16	74-83	—
	1202	52	6.4	15	—	57
	1086-1354	43-59	4.2-7.1	14-17	—	50-77
U.S.A.*	1000-1300	36-40	6.5-7.1	14-18	80	—
	1000-1300	37-41	6.5-7.3	11-15	—	65-72
England† 48	1305±9	62±5.6	15±1.4	14±2.1	80±1.5	—
23	1252±17	63±11	15±1.7	14±2.1	—	72±16

*Measurements of Thorne (1945), specimens from teasel *(Dipsacus sativus)*, U.S.A.
† Measurements of Blake (1962), specimens from oats *(Avena sativa)*, England.

Morphology

The following description and illustrations (Figs 1, 2, 3) are based on specimens from the four places;

Female: Body tapered anteriorly and posteriorly; tail tapered to a sharply pointed terminus. Lip region lightly sclerotized, smooth in light microscopy but striae apparent in SEM (Fig. 3), slightly flattened, barely offset, 6.9 μm (6-8) wide by 2.8 μm (2-4) (n = 5) high. Amphid openings slit-like (Fig. 3), on lateral lips. Body striae about 1 μm apart. Lateral field with four prominent lines, as in Fig. 1D, phasmids obscure. Spear slender with well defined knobs, 10-12 μm long. Excretory pore 140-160 μm from anterior end opposite middle of basal oesophageal bulb, hemizonid 8-12 μm anterior to excretory pore, 5-6 μm long. Nerve ring at middle of isthmus.

Median oesophageal bulb fusiform, 20 μm (17-26) by 11 μm (10-12) (n = 10). Isthmus narrow, widening posteriorly into clavate basal bulb containing three prominent and two inconspicuous gland nuclei. Basal bulb abuts squarely upon the intestine or overlaps it slightly. Intestine not extending into tail, a distinct rectum leads to anus.

Genital tract prodelphic, extending one third to half body length, ovary outstretched, oocytes in single file along distal third, then two rows changing to single file near proximal end. Oviduct (*sensu* Geraert, 1976) a short tube, 10 μm long, between ovary and spermatheca and consisting of four to six rings of cells, two cells in each ring (Fig. 2A). Spermatheca made up of large cells which become stretched when spermatheca is distended. Prominent quadricolumella behind spermatheca, composed of 4 rows each of 4 large cells (Fig. 2B) and about half as long as

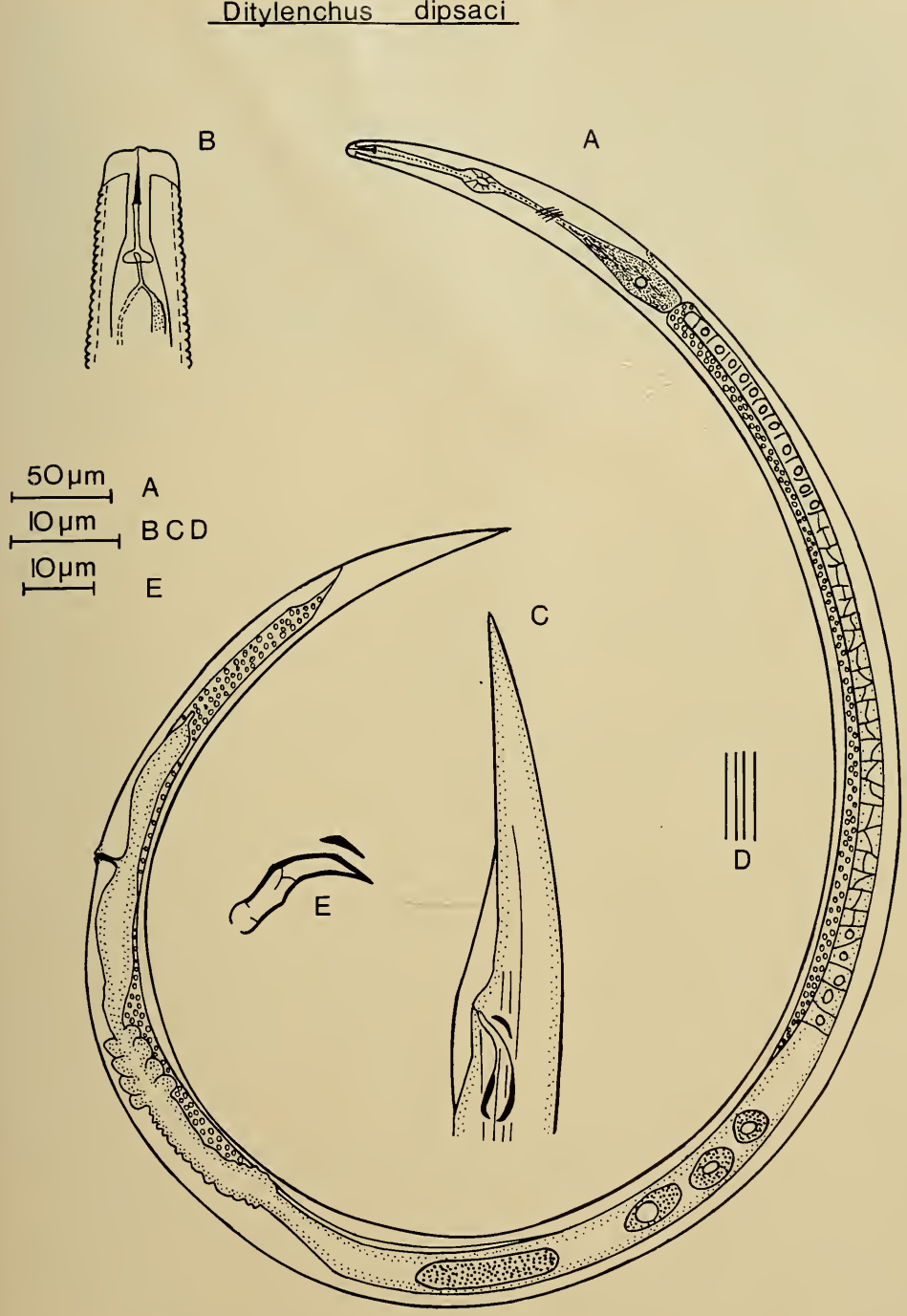

Fig. 1. Ditylenchus dipsaci. A. female, B. anterior end of female, C. male tail, D. lateral field, E. spicule and gubernaculum.

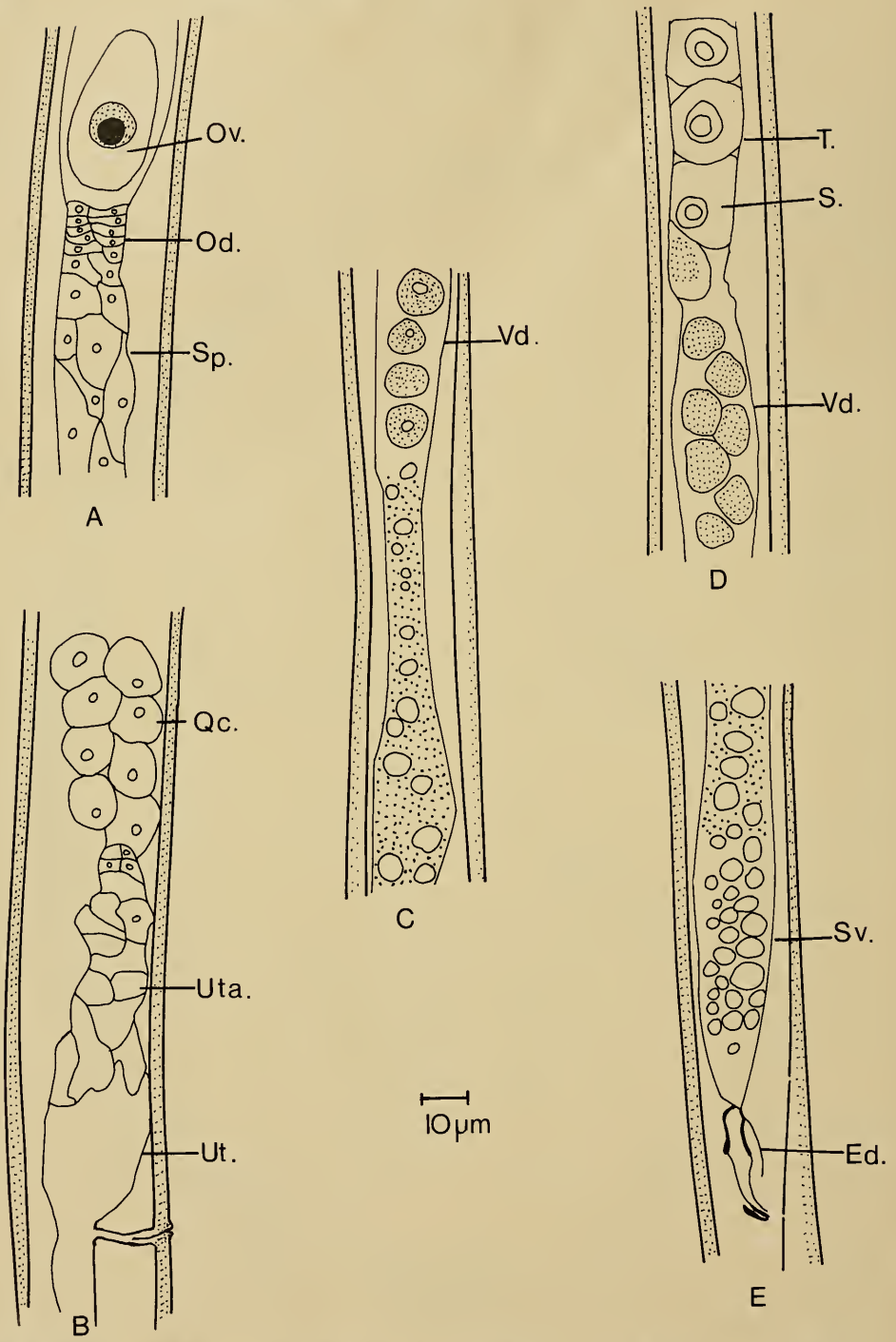

Fig. 2. Reproductive tracts of *Ditylenchus dipsaci*. **A.** proximal end of ovary, **B.** uterus, **C.** proximal end of vas deferens. **D.** proximal end of testis. **E.** proximal end of seminal vesicle. Ov. = ovary; Od. = oviduct; Sp. = spermatheca; Qc. = quadricolumella; Uta. = uterus, anterior part; Ut. = uterus; Vd. = vas deferens; T. = testis; S. = spermatocyte; Sv. = seminal vesicle; Ed. = ejaculatory duct.

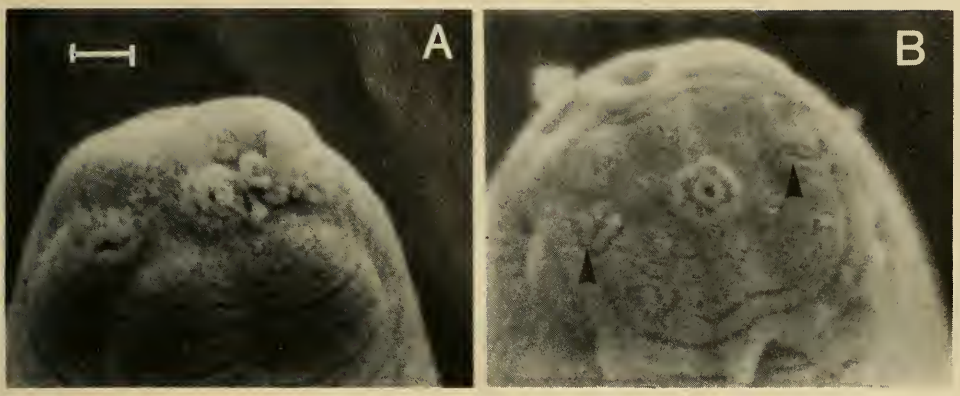

Fig. 3. Scanning electron micrograph of head-on view of *Ditylenchus dipsaci*. A. striation of lip region, B. amphid openings (arrowed). Bar = 1 μm. Specimens were dried in acetone followed by critical-point drying and coating with gold.

spermatheca (Fig. 1). Uterus with two distinct areas, an anterior slender part with a wall of thick small cells and a wide, thin walled posterior section. Eggs two to three times as long as wide. Minute epiptygmata in distal part of vagina as observed by Natasasmita and De Grisse (1976). Vulva a transverse slit. Posterior uterine sac approximately three anal body widths long, a terminal vestigial posterior ovary composed of two or three cells often present.

Male: Body bow-shaped when relaxed. Lip region flattened, slightly offset, 7.6 μm (7-8) wide by 3 μm (2.5-3.3) high (n = 5). Body tapered anteriorly and posteriorly, tail tapering to a sharply pointed terminus. Excretory pore 110-160 μm from anterior end, hemizonid as in female. Bursa begins in region of anterior one third of spicules and extends three-quarters of tail length.

Testis extends to middle of body, occupying 50% of length of genital tract. Spermatocytes in single row at distal and proximal ends and in two or three rows in the central multiplication region. Vas deferens same width as testis (Fig. 2D), occupying 20% of length of tract. Seminal vesicle (Fig. 2E) a dilated tube occupying about 30% of length of tract. A narrow ejaculatory duct, three quarters to one body width in length, extends posteriorly from the seminal vesicle but its junction with the cloaca could not be seen. Spicules shaped as in Fig. 1E, 21 μm (18-27, n = 48) long; gubernaculum lens shaped.

Distribution in New South Wales

The known distribution of *D. dipsaci* on lucerne in New South Wales (Noble, 1925; Edwards, 1932; Anon., 1949-1978; McLeod, 1979) is shown in Fig. 4.

Lucerne growing districts in New South Wales where *D. dipsaci* had been found were surveyed in the springs of 1976 and 1977. Within each district, crops were sampled at intervals of approximately 10 km and five lucerne crowns were collected at random from each crop sampled. Individual plants were placed in mist (Hooper, 1970) for 48hr to extract the nematodes. Measurements were made of soil pH, conductivity and texture in 1976.

Of the 79 crops sampled 17 (22%) were infested by *D. dipsaci* (Table 2). The pH range in soils where *D. dipsaci* occurred was only slightly more restricted than in soils

Fig. 4. Eastern part of N.S.W. showing recorded distribution of *Ditylenchus dipsaci* on lucerne in the State.

▲ indicates locality recorded in literature.
■ indicates *D. dipsaci* found in survey.

TABLE 2

Incidence of *Ditylenchus dipsaci* in lucerne crops in selected districts of N.S.W.

District	No. stands sampled	No. with *D. dipsaci*
Singleton (Hunter Valley)	10	4
Scone (Hunter Valley)	12	4
Inverell to Tamworth	23	3
Condobolin to Cowra (Lachlan Valley)	27	4
Richmond to Camden	7	2
Total	79	17 (22%)

apparently free of it while the range in conductivity was almost the same (Table 3). However, all soils where *D. dipsaci* occurred had 25% or more clay (Table 3).

Cross Inoculation of Lucerne and Daffodil Isolates

Attempts were made to inoculate *Narcissus pseudonarcissus* L. (daffodil) by injecting with a syringe *D. dipsaci* from Whittingham lucerne into bulbs. The inoculated bulbs were grown in pots for three months and then chopped and extracted in mist for 48 hr.

To determine whether *D. dipsaci* from daffodil invaded lucerne, 160 germinating lucerne seeds were inoculated with 40/seed *D. dipsaci* from daffodil bulbs from Bowral, N.S.W. and a similar number were inoculated with *D. dipsaci* from lucerne. Four days later the numbers of swollen seedlings were counted and the number of nematodes per seedling in 25 seedlings estimated after staining.

The daffodil bulbs were free of *D. dipsaci* 3 months after inoculation. 33% of lucerne seedlings inoculated with *D. dipsaci* from daffodil became swollen and contained an average of 1.6 nematodes/seedling. *D. dipsaci* from lucerne caused 92% swollen seedlings and there were an average of 30 nematodes/seedling.

TABLE 3

The relation between soil pH, conductivity and texture and the occurrence of *Ditylenchus dipsaci* in lucerne crops in N.S.W.

District	pH		Conductivity (mS/cm)		Texture*	
	Range, *D. dipsaci* absent	Range, *D. dipsaci* present	Range, *D. dipsaci* absent	Range, *D. dipsaci* present	Range, *D. dipsaci* absent	Range, *D. dipsaci* present
Singleton	6.9-7.5 (6)†	6.9-7.3 (4)	0.23-0.31 (6)	0.21-0.26 (4)	CL (6)	CL (4)
Scone	5.7-7.3 (8)	6.7-7.5 (4)	0.18-0.32 (8)	0.13-0.34 (4)	SCL-LMC (8)	LFS-LMC (4)
Inverell to Tamworth	5.6-8.1 (20)	6.7-7.7(3)	0.11-0.63 (20)	0.18-0.62 (3)	SL-HC (20)	LC-HC (3)

*SL = sandy loam, 10-15% clay content; SCL = sandy clay loam, 20-30% clay; LFS = Loam, fine sandy, 25% clay; CL = clay loam, 30-35% clay; LC = light clay, 35-40% clay; LMC = light medium clay, 40-50% clay; HC = heavy clay, 50% or more clay; (Northcote, 1971).
† Number of samples in this category.

Host Range of Lucerne Race

Hesling (1966) lists 21 races (differing in their host range) of *D. dipsaci*. Interbreeding between certain races has been demonstrated (Webster, 1967; Eriksson, 1974); the crosses may combine host range characteristics of the parents. Some strains, including the lucerne race, have a high degree of reproductive isolation (Eriksson, 1974).

Most observations overseas indicate that, under field conditions, the lucerne strain of *D. dipsaci* does not readily infest other hosts (Brown and Goodey, 1956; Thorne, 1961; Bingefors, 1969). In pot experiments Griffin (1975) showed that a lucerne strain of *D. dipsaci* from the western United States could not reproduce on *Allium cepa* L., *Beta vulgaris* L., *Lycopersicon esculentum* Mill., *Melilotus indica* (L.) All., *Triticum durum* Desf., but stunted, distorted or killed seedlings of these hosts. Sturhan (1975) found that a lucerne strain reproduced to a limited extent on only six of 23 varieties of *Vicia faba* L. whereas other races produced large populations on most varieties. On the other hand, Barker and Sasser (1959) found 14 out of 36 plant species were susceptible or slightly susceptible to two populations of *D. dipsaci* from lucerne from North Carolina.

In Australia *D. dipsaci* has been recorded on *A. cepa* (Cobb, 1891); *Ceratochloa unioloides* HBK. (Edwards, 1932); *Hyacinthus orientalis* L. (Hynes *et al.*, 1941); *Hyacinthus romanus* L. (Anon., 1977); *L. esculentum* (Anon., 1941); *Medicago polymorpha* L., *M. minima* (L.) Bartal. (Edwards, 1932); *M. sativa* (Noble, 1925); *Narcissus jonquilla* L., *N. pseudonarcissus* L. (Noble, 1928); *Phaseolus vulgaris* L. (Wilson, 1942); *Phlox drummondii* Hook. (Hynes *et al.*, 1941); *P. paniculata* L., (Anon., 1941); *Trifolium pratense* L., (Noble *et al.*, 1937); *T. repens* L. (Anon., 1955); *V. faba* (Anon., 1941).

The infested plants of *C. unioloides, M. polymorpha* and *M. minima* were found amongst heavily infested lucerne plants (Edwards, 1932) hence it is probable that they were infested with the same race of nematode as the lucerne.

The aim of this work was to find whether the strain of *D. dipsaci* attacking lucerne in New South Wales has a narrow or a wide host range.

Materials and Methods

Two experiments were done, one with nematodes from Whittingham and the second with nematodes from Scotts Flat. Two lots of seed of plants listed in Table 4 were germinated on filter paper in Petri dishes. Immediately after germination a suspension of adults and larvae of *D. dipsaci* was added to one dish of each host, so as to add 50 nematodes/seed. A 15 cm diameter pot was then planted with five uninfested seeds and another four planted with five infested seeds. Pots were kept covered with plastic sheet for 48 hr after planting to maintain humidity. Growth abnormalities were noted 3 weeks after planting. Ten weeks after inoculation in the first experiment and 4 weeks after in the second experiment, the tops of the ten most affected plants were cut and placed in mist for 48 hr to extract nematodes present.

Results

Results are given in Table 4. The nematodes caused stunting, twisting of the stem and leaf puckering of *L. lycopersicon* (tomato), *P. vulgaris* (French bean) and *Pisum sativum* L. (pea). On *A. cepa* (onion) the symptoms were twisting and tip necrosis of the leaves and many seedlings were killed. Lucerne seedlings showed the typical swelling of the hypocotyl region. Nematodes were recovered from tissues of plants other than lucerne only in the second experiment, when extraction was done 4 weeks after planting.

TABLE 4

Numbers of nematodes in and reactions of plants following inoculation* with *Ditylenchus dipsaci*

Plant	Source of *D. dipsaci*			
	Whittingham		Scotts Flat	
	Reaction	No. *D. dipsaci*‡	Reaction	No. *D. dipsaci*#
Allium cepa				
c. v. Hunter River White	+ †	0	+	270
Avena sativa				
c. v. Acacia	—	0	—	0
c. v. Algerian	—	0	—	0
c. v. Avon	—	0	—	0
Cynodon dactylon	—	0	—	0
Lycopersicon esculentum				
c. v. Grosse Lisse	+	0	+	470
Mathiola incana				
c. v. Giant Perfection	—	0	—	0
Medicago sativa				
c. v. Hunter River	+	570	+	800
Medicago trunculata				
c. v. Cyprus	—	0	—	0
Phaseolus vulgaris				
c. v. Stringless Tender Crop	+	0	+	10
c. v. Blue Lake	+	0	+	220
Pisum sativum				
c. v. Massey Gem	+	0	+	280
Secale cereale				
c. v. Strain 8	—	0	—	0
Trifolium alexandrinum	—	0	—	0
Trifolium pratense				
c.v. Cowgrass	—	0	—	0
Trifolium repens				
c. v. Louisiana	—	0	—	0
Tirolium suberraneum				
c. v. Mount Barker	—	0	—	0

* Germinating seedlings inoculated with 50 *D. dipsaci* to a seedling.
† + indicates stunting of plant or twisting of stem and leaf puckering, or, in *A. cepa,* tip necrosis of leaves and death of seedlings.
‡ Number of nematodes from tops of 10 plants, including any showing abnormal growth, 10 weeks after inoculation.
# Numbers of nematodes from tops of 10 plants, including any showing abnormal growth, 4 weeks after inoculation.

Discussion

Measurements of these Australian lucerne populations are close to those of *D. dipsaci* from the type host (Thorne, 1945) (Table 1). Blake's (1962) specimens of the oat race from oats were much thinner (a = 62± 5.6 for females and 63± 11.3 for males) and had shorter oesophagi (b = 15± 1.4 for females and 15± 1.7 for males). However, Metlitzki (1968, 1969) concluded that measurements and their ratios are useless for separating races within *D. dipsaci* and Hooper and Southey (1978) report that even measurements on the giant race and the oat race overlap.

The structures of the female and male genital tracts are as described for *D. destructor* and *D. dipsaci* by Wu (1958, 1967). Wu (1967) noted that in *D. dipsaci* the spermatheca is longer than the quadricolumella whereas they are of similar length in *D. destructor*. My observations of Australian *D. dipsaci* confirm this.

D. dipsaci was recovered from 22% of crops sampled within known infested districts. It is interesting that Adamova (1975) reports that 6% of plants in 23% of the lucerne growing areas of Czechoslovakia were severely infested. The distribution of *D. dipsaci* appeared not to be restricted by pH or soil conductivity within the ranges examined. In the Netherlands *D. dipsaci* causes severe damage to onions in soils containing 30% or more clay (Seinhorst, 1956). I found *D. dipsaci* only on soils with 25% or more clay. A requirement for heavy soils could explain why economic damage to lucerne in New South Wales occurs mainly in river valleys with heavy alluvial soils.

The results indicate that the *D. dipsaci* attacking *Narcissus* and lucerne in New South Wales are two distinct races, distinguishable by cross-inoculation. Barker and Sasser (1959) could not infest daffodils with *D. dipsaci* from lucerne in the U.S.A. and Webster (1967), in England, found that inoculation of daffodil race with a lucerne race and of lucerne with a daffodil race resulted in invasion but no multiplication or tissue reaction.

Failure to extract nematodes other than from lucerne after 10 weeks suggests that the nematode was unable to persist in those plants which showed growth distortion and contained nematodes 4 weeks after inoculation. It seems unlikely that two nematode strains are involved since they came from localities less than 2 km apart and they caused growth distortion on the same plants. Although it may be possible to demonstrate a wider range than obtained here by using special techniques (Webster, 1967), my results suggest that, under field conditions, the lucerne race of *D. dipsaci* in Australia would reproduce on few plants other than lucerne. It could, however, cause symptoms on and damage to onion, tomato, French bean and pea.

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